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LETTER

Genetic variability of Appaloosa horses: a study of a closed breeding population from Argentina

Claudia Malena CORBI-BOTTO (✉)[1,2], Sebastian Andres SADABA[1,3], Elina Ines FRANCISCO[1], Paula Belen KALEMKERIAN[1], Juan Pedro LIRON[1], Egle Etel VILLEGAS-CASTAGNASSO[1], Guillermo GIOVAMBATTISTA[1], Pilar PERAL-GARCIA[1], Silvina DIAZ[1]

1 Instituto de Genética Veterinaria "Ing. Fernando N Dulout"(IGEVET) –CCT La Plata –CONICET, Facultad de Ciencias Veterinarias, Universidad Nacional de La Plata, La Plata, Argentina
2 Research Fellow from Consejo Nacional de Invetigaciones Cientificas y Tecnicas (CONICET), Argentina
3 Research Fellow from Universidad Nacional de la Plata (UNLP), Argentina

Abstract The genetic diversity and structure of 72 Appaloosa horses belonging to a closed breeding population from an ecological reserve in Buenos Aires, Argentina, was investigated using eight microsatellite markers from the International Society for Animal Genetics panel. Our data showed that this Appaloosa horse population had an elevated degree of genetic diversity (He = 0.746) and did not present a significant increase of homozygous individuals (F_{IS}~0). However, the short tandem repeats, AHT5, ASB2, HTG10 and VHL20, were not in Hardy–Weinberg equilibrium (P-value < 0.05). Genetic relationships between this population and other well known horse breeds showed that Appaloosa horses from Argentina could have had their origin in the horses of the Nez Perce's people in Idaho while other Appaloosa horses may have had influences from Andalusian and Lusitano breeds. This closed breeding population conserves an important degree of Appaloosa genetic diversity and notwithstanding its particular breeding characteristics, represents a valuable genetic resource for conservation.

Keywords horse, genetic diversity, microsatellite, Appaloosa, population structure, conservation

1 Introduction

The first trace of what is today known as the Appaloosa horse goes back to prehistoric times. These horses were introduced to America during the Spanish colonization; years later, the Nez Perce people of Idaho developed a special interest in Appaloosa horses. Pleased with their characteristics such as distinctive spotted coats, versatility and robustness, the Nez Perce only bred the ones they considered to be the best for hunting, racing and war.

The present study arose from the interest of an Appaloosa stud farm owner to evaluate the level of consanguinity of his horses kept on an ecological reserve near Buenos Aires, Argentina. The reserve had about a hundred horses, originating from the crossbreeding of a few individuals acquired as pure breed Appaloosa. Since genetic characterization of breeds is a compelling prerequisite for preservation and management strategies [1,2], this study was conducted to quantify genetic variation within a closed breeding population of Appalossa horses and to compare this with a selection of breeds, including Appaloosa, from different origins.

2 Materials and methods

Genomic DNA was isolated from blood samples from 72 Appaloosa horses. Eight microsatellites (short tandem repeat loci, AHT4, AHT5, ASB2, HMS3, HMS6, HTG4, HTG10 and VHL20) as recommended by the International Society for Animal Genetics [3] were amplified by PCR in an MAXYGENE instrument (Axygene Inc., Union City, CA). Genotypes were determined on a MegaBase 1000 automated sequencer (GE Healthcare, Buckinghamshire, UK) using ET550-Rox as molecular size standard (GE Healthcare) and assessed by using Fragment Profiler Software Suit version 2.2 (MegaBase Build 1.2.0311.2500, Amersham Biosciences, GE Healthcare, Buckingamshire, UK, 2003).

Population genetic parameters for the Appaloosa horses from Argentina were estimated by GENEPOP [4]. For Hardy–Weinberg equilibrium, P-values less than 0.05 were considered significant. To assess the distribution of

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Correspondence: ccorbi@fcv.unlp.edu.ar

the genetic variability within and among breeds, a comparative analysis was performed by using microsatellite diversity from the following eight breeds [5]: Appaloosa, Arabian, Thoroughbred, Andalusian, Haflinger, Dutch, Lusitano and Standardbred.

3 Results

The genetic variability detected in this study (Table 1) was very similar to that found in Appaloosa by Van der Goor [5]. Overall, the nine populations showed high levels of heterozygosity, ranging from 0.674 for Dutch to 0.771 for Lusitano. Appaloosa from Argentina showed a higher F_{IS} value (0.067), indicating a deficit of heterozygotes, likely reflecting the lack of reproductive management of the stud farm. The number of markers not in Hardy–Weinberg equilibrium ranged from 0 in Standardbred and Dutch, to 4 (AHT5, ASB2, HTG10 and VHL20) in Appaloosa from Argentina.

Based on the genotypes of the eight short tandem repeats loci, individuals were clustered into a given number of populations and assigned probabilistically to two to eight possible clusters (K) inferred with the Bayesian approach of STRUCTURE 2.3.4 [6]. The proportional membership of individual genotypes in different clusters (Fig. 1) indicates that, for K = 2, one cluster included Arabian, Thoroughbred, Standardbred, Andalusian and Lusitano; another one the Haflinger and Dutch horses; while both Appaloosa populations showed some level of admixture with a particular inverted pattern (Fig. 1). For K = 4, Appaloosa horse from Argentina and American Standardbred were assigned to the same genetic cluster, Arabian and Thoroughbred fell into a second cluster; whereas the Appaloosa population conformed to a third cluster with Andausian and Lusitano horses. Finally, the fourth cluster was composed Haflinger and Dutch horses (Fig. 1). The relationship between Thoroughbred and Arabian [7] as well as the link between Andalusian and Lusitano has been reported previously [7,8].

4 Discussion

Increased homozygosity as a consequence of inbreeding in a closed population could represent a disadvantage for the whole population if it concentrates no beneficial and recessively transmitted characters. The breeding practices exercised by the horse owner, may further weaken the diversity levels through the breeding between relatives, increasing the probability of recessive diseases occurrence.

Some methods have recently been developed to evaluate the genetic contribution of populations to within-breed and between-breed diversities [9,10]. In the last decades, microsatellite markers have been used to evaluate genetic distances and to characterize local breeds [11,12].

It has been suggested [13,14] that the population sizes of various horse breeds declined appreciably in the 19th and early 20th centuries. Although a reduction in variability might have been expected in the Appaloosa from Argentina, no such significant effect was detected in our study (Table 1). As discussed in [15] bottlenecked populations might not show distorted allelic distribution for several reasons such as size of the sample or polymorphism level of the loci studied, the representativeness of sampled individuals, the possible occurrence of a demographic but not genetic bottleneck, and the presence of immigrants genes in a partially isolated population [14].

5 Conclusions

The biologic unit for conservation in domesticated animals is usually the breed. Obtaining information from molecular

Table 1 The genetic variability of 9 horse populations

Population	ID	N	Heterozygosity		Na	F_{IS}	HWE*	Reference
			Expected	Observed				
Appaloosa (ARG)	AP	72	0.746	0.697	7.63	0.066	4	This study
Appaloosa	APP	99	0.765	0.761	7.63	0.005	2	[5]
Arabian	ARA	100	0.676	0.666	6.50	0.015	1	[5]
Thoroughbred	THO	54	0.713	0.698	5.25	0.021	1	[5]
Andalusian	AND	67	0.721	0.711	6.88	0.015	4	[5]
Haflinger	HAF	65	0.711	0.690	5.50	0.015	1	[5]
Dutch	DUT	73	0.674	0.724	6.00	−0.023	0	[5]
Lusitano	LUS	43	0.771	0.788	6.63	0.029	1	[5]
Sandardbred	STA	100	0.749	0.738	6.63	−0.075	0	[5]

Note: N: number of individuals per population; Na: number of alleles; F_{IS}: within-population inbreeding coefficient; HWE: number of loci with Hardy–Weinberg equilibrium; *: $P < 0.05$.

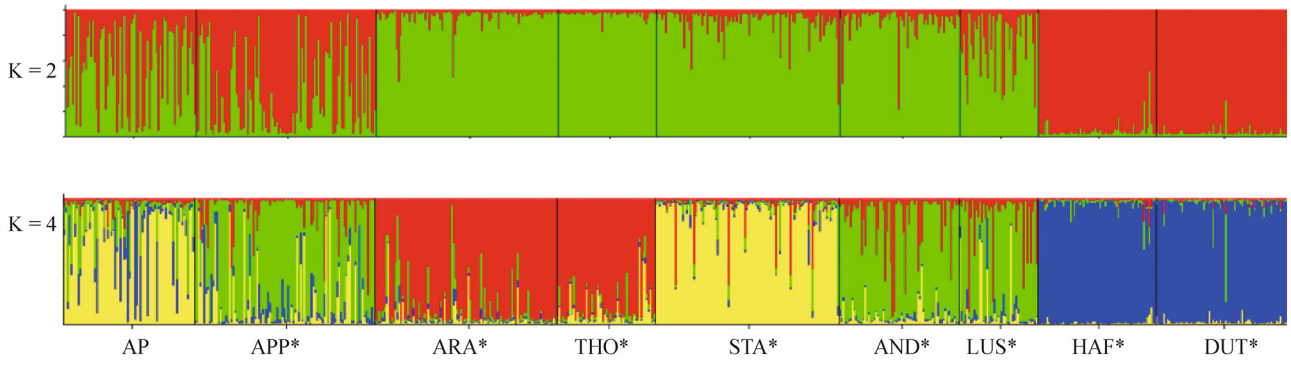

Fig. 1 Model-based clustering of 9 horse populations using STRUCTURE software. The 8 STR loci genotypes were analyzed using an admixture model with a burnin length of 100000 followed by 1000000 Markov Chain Monte Carlo (MCMC) replicates. Each animal is represented by a single vertical line divided into K colors, where K is the number of clusters assumed and the colors show the estimated individual proportions of cluster membership. Results are shown for (a) K = 2 and (b) K = 4. AP: Appaloosa Argentina; APP: Appaloosa; ARA: Arab; THO: Thoroughbred; STA: Standardbred; AND: Andalusian; LUS: Lusitan; HAF: Haflinger; DUT: Dutch Horse; *: Source [5].

markers made it possible to create a hypothetical scenario for assessing different methods of analyzing diversity for conservation [14]. The present study contributes to the knowledge of genetic diversity and population structure of the Appaloosa horse from Argentina. Genetic relationships between this population and other well known breeds, showed that Appaloosa horses from Argentina could have had their origin in the horses of the Nez Perce's people, while the other Appaloosa horses may have had some interbreeding with Andalusian and Lusitano breeds.

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All applicable institutional and national guidelines for the care and use of animals were followed.

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